Remarks

Claims 1, 3-21, 30-34, 36, and 37 are pending in the subject application. By this Amendment, Applicant has amended claim 1 and canceled claims 18-20. Support for the amendments can be found throughout the subject specification and in the claims as originally filed, including, for example, canceled claim 18. Applicant respectfully asserts that the amendments presented herein will not require any further search on the part of the Examiner. Entry and consideration of the amendments is respectfully requested. Accordingly, claims 1, 3-17, 21, 30-34, 36, and 37 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

Claims 1, 3-9, 21, 30-34, 36, and 37 are rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Seeger (U.S. Patent No. 5,360,714). In addition, the claims are also rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Seeger (U.S. Patent No. 5,360,714) further in view of each of Schwarz *et al.* (1991), Chang *et al.* (U.S. Patent No. 5,801,042), O'Donnell (U.S. Patent No. 6,221,642), Rosenthal *et al.* (WO 93/21340), Vind (U.S. Patent No. 6,159,687), and Smith *et al.* (U.S. Patent No. 5,753,439). Applicant respectfully traverses each of these grounds of rejection.

Applicant respectfully asserts that the subject invention is not obvious over any combination of the cited references. However, in a sincere effort to advance prosecution of the subject application to completion, Applicant has amended step (ii) in claim 1. Support for this amendment can be found, for example, at page 3, lines 36-37, through to page 4, lines 1-2, of the subject specification. The amendments to step (ii) make it clear that what is detected is the interaction between these distinct elements: the polymerase enzyme, the target polynucleotide, and a nucleotide complementary to a nucleotide in the target polynucleotide. This clearly distinguishes the claimed invention from the method disclosed in the cited references, including the Tsien *et al.* publication, where the detection is carried out after the incorporation of the nucleotide onto the target polynucleotide (the polymerase enzyme no longer being present) and it is a label on the incorporated nucleotide that is detected. The amendment to step (ii) in claim 1 also specifies that detection is carried out using surface plasmon resonance (SPR) to measure a change in, or absorption of,

radiation that occurs during the interaction. Support for this amendment can be found, for example, in canceled claim 18 which depended from claim 1.

Applicant respectfully maintains that the disclosure in the Tsien *et al.* reference is inconsistent with the requirement in the claims of the subject application directed to detecting the interaction of the various reaction components. Tsien *et al.* disclose a DNA sequencing procedure carried out by measuring the stepwise incorporation of labeled nucleotides complementary to a target polynucleotide. There is an explicit requirement for the target polynucleotide to be immobilized (see in particular page 32, lines 9-36, and all of pages 33-34 of the Tsien *et al.* reference). There is also an explicit requirement for the nucleotides to comprise blocking groups, so that only one nucleotide can be incorporated at any one time. Detection of the incorporated nucleotide occurs only after the non-incorporated components, including the (unbound) polymerase, are removed during a washing step. Additional nucleotide incorporations can follow upon removal of the blocking group and upon the reintroduction of the various nucleotides and the polymerase back into the reaction chamber (see page 13, lines 23-29 of the Tsien *et al.* reference).

In contrast to the methods disclosed in Tsien *et al.*, the methods of the present invention are carried out to detect the interaction between the polymerase enzyme, the target polynucleotide, and a specific nucleotide complementary to the target polynucleotide. The interaction can be detected from the measurement of the change in, or absorption of, radiation that occurs during the interaction. As noted previously in Applicant's remarks, claim 1 has been amended to recite that SPR is used as the means for detecting a change in, or absorption of, radiation. In addition, by using SPR as the detection means, nucleotides used in Applicant's claimed methods do not have to be labeled.

A specific element of the claimed invention is that the polymerase enzyme is immobilized. Although the Examiner appears to be of the opinion that this would be an obvious alternative (citing Holzrichter *et al.*) to immobilizing the DNA, Applicant respectfully stresses that if the polymerase enzyme is immobilized, rather than the target polynucleotide, then the method of Tsien *et al.* **will not work**. It is well accepted in patent law that if a proposed modification of the prior art in an effort to attain the claimed invention causes the art to become inoperable or destroys its intended function, then the requisite motivation to make the modification would not have existed. See *In re Fritch*, 23 USPQ2d 1780, 1783 n.12 (Fed. Cir. 1992) ("A proposed modification [is] inappropriate for an

obviousness inquiry when the modification render[s] the prior art reference inoperable for its intended purpose."). As stated above, the Tsien *et al.* method requires that the nucleotides are blocked, to prevent further nucleotide incorporation and nascent strand synthesis. Blocking the nucleotides has the effect of stopping the polymerase reaction (which is necessary in the Tsien *et al.* method to permit detection of the label), and in doing so, the polymerase disassociates from the target polynucleotide. The washing step in the Tsien *et al.* method (see, for example, page 12, lines 29-34 of the Tsien *et al.* reference) washes away all the non-incorporated nucleotides and the polymerase from the reaction chamber. Accordingly, if the skilled artisan were to modify the method of Tsien *et al.* so as to immobilize the polymerase (rather than the target polynucleotide), the result would be that the target polynucleotide would dissociate from the polymerase following incorporation of the blocked nucleotide and then would be removed from the reaction chamber during the washing step. It would then be impossible to detect the incorporation event because the nascent strand with the incorporated labeled nucleotide is washed away. The proposed modification would effectively destroy the intended purpose of the method disclosed in the Tsien *et al.* reference. Moreover, the ordinarily skilled artisan would know that these problems would result from modifying the Tsien *et al.* method to immobilize the polymerase. Therefore, it would be inconceivable for the skilled artisan to modify, or even consider modifying, the teaching of Tsien *et al.* in the manner suggested by the Examiner.

In addition to the distinctions over the cited references discussed in the preceding paragraphs of this Amendment, Applicant has amended claim 1 to incorporate the elements of claim 18, *i.e.*, to specify that the interaction is detected using surface plasmon resonance. In making the rejection of claim 18, which specified SPR as the detection methodology, the Examiner cited (in addition to the primary references) the publication by Schwarz *et al.* as teaching "the detection of nucleic acid incorporation by surface plasmon resonance signal over time in the infra-red spectrum (Figure 2 and Page 340, Columns 1-3)." (emphasis added). Applicant respectfully asserts that the Schwarz *et al.* reference does **not** teach or suggest anything concerning detection of the incorporation of a nucleotide into a nascent polynucleotide being synthesized during a polymerase reaction in DNA sequencing methods, or the interaction between the polymerase, the target polynucleotide, and the complementary nucleotide. The Schwarz *et al.* reference only teaches the use of SPR to detect

hybridization of a nucleic acid strand to another nucleic acid strand (note the title of the Schwarz *et al.* reference: "Detection of Nucleic Acid Hybridization Using Surface Plasmon Resonance") (emphasis added). The determination of a sequence of a DNA molecule is for the most part unrelated to nucleic acid hybridization and the detection of the same. There is no teaching or suggestion in the Schwarz *et al.* reference regarding the use of SPR to detect incorporation of a single nucleotide into a nascent polynucleotide strand being synthesized, or the interaction between the polymerase, the target polynucleotide, and the complementary nucleotide. Moreover, there is no teaching or suggestion in any of the cited references as to how one might modify the SPR technique used in hybridization experiments/assays as disclosed in Schwarz *et al.* for use in a DNA sequencing method as claimed in the subject application. Applicant also notes that the Schwarz *et al.* reference teaches that the polynucleotide is immobilized on a solid surface. Accordingly, Applicant respectfully asserts that the Schwarz *et al.* reference does not cure the deficiencies of the primary references and, therefore, the combination of references cited by the Examiner does not render obvious Applicant's claimed invention.

In view of the above remarks, reconsideration and withdrawal of the rejections under 35 USC §103 is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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DRP/sl
Attachment: Marked-Up Version of Amended Claim

Docket No. GJE-35
Serial No. 09/463,549

Marked-Up Version of Amended Claim

Claim 1 (thrice amended):

1. A method for sequencing a polynucleotide, comprising the steps of:

(i) reacting a target polynucleotide with a polymerase enzyme immobilised on a solid support, and complementary nucleotides, under conditions sufficient for the polymerase reaction; and

(ii) detecting [an effect consequent on the incorporation of] the interaction between the polymerase, the target polynucleotide and a specific nucleotide complementary to the target polynucleotide, wherein the nucleotide is incorporated into a nascent polynucleotide being synthesized as a result of the polymerase reaction, to thereby determine the sequence of the target polynucleotide, the detection being carried out by [measuring] using surface plasmon resonance to measure a change in, or absorption of, radiation that occurs [if the nucleotide is incorporated] during the interaction.